

What is claimed:

1. A method for amplifying a target nucleic acid sequence comprising subjecting a sample of biological material containing a target nucleotide sequence to  
5 amplification using oligonucleotide primers and blockers to create primer extension products that are susceptible to cleavage by double-strand-specific ribonucleases wherein continuous production and cycling of ribonuclease cleaved products allows for amplification of said target sequence.
- 10 2. The method of claim 1 wherein said double-strand-specific ribonuclease is thermostable RNaseH.
3. A method for amplifying a target nucleic acid sequence comprising the steps of;
  - 15 a) forming a nucleotide amplification reaction mixture comprising a DNA template containing a target nucleic acid sequence; a single chimeric oligonucleotide primer consisting of a deoxyribonucleotide sequence with a ribonucleotide base at the 3' terminus that binds to said DNA template; a non-extendable oligonucleotide blocker that binds to said DNA template downstream from said primer; a DNA polymerase which  
20 lacks 5' exonuclease activity; and a double-strand-specific ribonuclease, and appropriate buffers and nucleic acid precursors;
  - b) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a first primer extension product is formed and  
25 cleaved at the ribonucleotide base releasing said first primer extension product;
  - c) hybridizing said first primer extension product to a first DNA triggering template comprising a target sequence, a first primer extension product binding site at the 3' terminus of said target sequence, and a contiguous second primer sequence which is conjoined to the 5'  
30 end of said target sequence by a ribonucleotide base;
  - d) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a target amplification product is formed and

said first DNA triggering template is cleaved at the ribonucleotide base releasing said second primer sequence with a ribonucleotide base at the 3' terminus;

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e) hybridizing said second primer sequence to a second DNA triggering template which contains a second primer sequence binding site at the 3' terminus; and

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f) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a third primer extension product is formed and cleaved at the ribonucleotide base releasing said third primer extension product, wherein said third primer extension product has the same nucleotide sequence as the first primer extension product;

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4. The method of claim 3 wherein said DNA polymerase is the Stoffell fragment of Taq polymerase and has strand displacement activity.

5. The method of claim 3 wherein said double-strand-specific ribonuclease is thermostable RNaseH.

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6. The method of claim 3 wherein said thermocycle includes a hybridization step at a temperature in the range of 30 to 50 degrees Celsius, a primer extension step at a temperature in the range of 50 to 70 degrees Celsius, and a double-strand-specific ribonuclease cleavage step at a temperature in the range of 50 to 70 degrees Celsius.

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7. The method of claim 3 wherein said thermocycle is begun at a temperature in excess of 85 degrees Celsius.

8. The method of claim 3 wherein said nucleotide amplification reaction mixture is a polymerase chain reaction mixture.

9. The method of claim 3 wherein said nucleotide amplification reaction mixture includes a molar excess of said first and second DNA triggering templates over said DNA template.

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10. A method for amplifying a target nucleic acid sequence comprising the steps of;
- a) forming a nucleotide amplification reaction mixture comprising a DNA template; a single chimeric oligonucleotide primer consisting of a deoxyribonucleotide sequence with a ribonucleotide base at the 3' terminus that binds to said DNA template; a non-extendable oligonucleotide blocker that binds to said DNA template downstream from said primer; a DNA polymerase which lacks 5' exonuclease and strand displacement activity; a double-strand-specific ribonuclease; and appropriate buffers and nucleic acid precursors
  - b) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a first primer extension product is formed and cleaved at the ribonucleotide base releasing said first primer extension product;
  - c) hybridizing said first primer extension product to a first DNA triggering template comprising a target sequence, a first primer extension product binding site at the 3' terminus of said target sequence, and a contiguous second primer sequence which is conjoined to the 5' end of said target sequence by a ribonucleotide base;
  - d) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a target amplification product is formed and said first DNA triggering template is cleaved at the ribonucleotide base releasing said second primer sequence with a ribonucleotide base at the 3' terminus;
  - e) hybridizing said second primer sequence to a second DNA triggering template which contains a second primer sequence binding site at the 3' terminus;

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- f) subjecting said nucleotide amplification reaction mixture to at least one thermocycle such that a third primer extension product is formed and cleaved at the ribonucleotide base releasing said third primer extension product, wherein said third primer extension product has the same nucleotide sequence as the first primer extension product;
  - g) repeating steps c)-f);and
  - h) detecting amplification of said target sequence.
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11. The method of claim 10 wherein said DNA polymerase is the Stoffell fragment of Taq polymerase.
12. The method of claim 10 wherein said double-strand-specific ribonuclease is thermostable RNaseH.
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13. The method of claim 10 wherein said thermocycle includes a hybridization step at a temperature in the range of 30 to 50 degrees Celsius, a primer extension step at a temperature in the range of 50 to 70 degrees Celsius, and a double-strand-specific ribonuclease cleavage step at a temperature in the range of 50 to 70
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- degrees Celsius.
14. The method of claim 10 wherein said thermocycle is begun at a temperature in excess of 85 degrees Celsius.
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15. The method of claim 10 wherein said nucleotide amplification reaction mixture is a polymerase chain reaction mixture.

16. The method of claim 10 wherein said target product in step d) is labeled with a detectable marker and said labeled target product is detected in step h).

5 17. The method of claim 10 wherein said target product in step d) is contacted with a DNA chelating agent and said target product is detected in step h).

18. The method of claim 10 wherein said nucleotide amplification reaction mixture includes a molar excess of said first and second DNA triggering templates over said DNA template.

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19. A kit for amplifying a target nucleic acid sequence comprising;

15 a nucleotide amplification reaction mixture contained in one or more containers comprising a DNA template; a single chimeric oligonucleotide primer consisting of a deoxyribonucleotide sequence with a ribonucleotide base at the 3' terminus that binds to said DNA template; a non-extendable oligonucleotide blocker that binds to said DNA template downstream from said primer; a DNA polymerase which lacks 5' exonuclease and strand displacement activity; and a double-strand-specific ribonuclease; a first DNA triggering template comprising a target sequence, a first primer extension product binding site at the 3' terminus of said target sequence, and a  
20 contiguous second primer sequence which is conjoined to the 5' end of said target sequence by a ribonucleotide base; a second DNA triggering template which contains a second primer sequence binding site at the 3' terminus; appropriate buffers and nucleic acid precursors.

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20. The method of claim 19 wherein said DNA polymerase is the Stoffell fragment of Taq polymerase.

21. The method of claim 19 wherein said double-strand-specific ribonuclease is thermostable RNaseH.
- 5 22. The method of claim 19 wherein said nucleotide amplification reaction mixture is a polymerase chain reaction mixture.
23. The method of claim 19 wherein said nucleotide amplification reaction mixture includes a molar excess of said first and second DNA triggering templates over  
10 said DNA template.